Application Manual

Instruction Manual

FastDNA-96™ Soil Microbe DNA Kit

One Call

Rapid, High-Throughput Isolation of PCR - Ready Genomic DNA from Soil Samples using the FastPrep-96™ System

One Source

Catalog # 9696-200
2 x 96 Preps

A World of Biotechnology Reagents

Storage:
Ambient temperature (15 – 30°C)

Revision # 9696-200-11FEB

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TABLE OF CONTENTS

1. Introduction to the FastDNA-96™ Soil Microbe DNA Kit and the FastPrep-96™ Instrument 5
2. Kit Components, Storage, & User Supplied Materials 6
2.1 FastDNA-96™ Soil Microbe DNA Kit Components 6
2.2 Storage 7
2.3 User Supplied Materials 7
3. Important Considerations Before Use 7
3.1 Binding Plate Pre-Wash Buffer 7
3.2 MP-96 Inhibitor Removal Plate Preparation 8
4. Safety precautions 8
5. Protocol 8
6. Example Data: DNA Isolation from Soil Samples and Gel Electrophoresis 11
7. Recommended Reference Format for Publication 12
8. References 12
9. Technical Support 12
10. Product Use Limitation & Warranty 13

1. Introduction to the FastDNA-96™ Soil Microbe DNA Kit and the FastPrep-96™ Instrument

The FastDNA-96™ Soil Microbe DNA Kit quickly and efficiently isolates humic-free, PCR-ready genomic DNA from soil samples in approximately 50 minutes. Gram(+/-) bacteria, fungi, plant and animal tissues, algae, spores and many other members of a soil population including clay, sandy, silty, peaty, chalky, and loamy soils, are efficiently lysed in approximately 60 seconds with the FastPrep-96™ Instrument from MP Biomedicals.

The FastPrep-96™ Instrument is a high-throughput homogenizer developed to disrupt thoroughly any tissues and cells through the simultaneous bead-beating and impaction of specialized Lysing Matrix beads on the sample material. The FastPrep-96™ Instrument uses a linear vertical bidirectional motion providing an extremely quick and highly reproducible homogenization that surpasses early generation homogenizers, as well as traditional extraction methods using enzymatic digestion, sonication, blending, douncing or vortexing.

Samples are placed into 1.2 ml size tubes of a FastDNA-96™ Lysing Matrix Rack (96-deep well plate) containing 0.5 mm lysing matrix beads. The lysing matrix beads, which are specially stabilized Zirconium oxide particles, are designed to efficiently lyse all soil organisms including historically difficult sources such as eubacterial spores and endospores, gram (+/-) bacteria, yeast, algae, nematodes, and fungi, while in the presence of a specially formulated lysis solution.

The FastDNA-96™ Soil Microbe DNA Kit isolates microbial DNA from up to 130mg of soil or sludge through a purification process that eliminates completely polyphenols and humic

acids. Organic denaturants or proteinases are not needed with this procedure. Alternatively, DNA can be isolated from bacterial or fungal broth cultures directly. Purified, humic-free DNA is eluted in an EDTA-free, DNA elution solution, and is ready for downstream applications including digestion, electrophoresis and PCR (A260/A280 ratios ≥ 1.8). Yield is typically 5 µg of total DNA eluted in 50-100 µl of elution solution.

The FastDNA-96™ Soil Microbe DNA Kit will recover genomic DNA fragments from 25 kb to 35 kb; however, some experiments have yielded results from as little as 100 bp up to >40 kb. Viral and parasitic DNA will also be isolated if these hosts are present in your samples.

2. Kit Components, Storage and User Supplied Materials

2.1 FastDNA-96™ Soil Microbe DNA Kit Components

FastDNA-96™ Lysing Matrix Rack (0.5 mm Beads)	2 x 96-well rack
Lysis Buffer	2 x 40 ml
Soil DNA Binding Solution	150 ml
Binding Plate Pre-Wash Buffer	50 ml
Soil DNA Wash Buffer	100 ml
Elution Solution	2 x 10 ml
Inhibitor Plate Prep Solution	30 ml
Deep-Well Plate	2 each
MP-96 Binding Plate	2 each
MP-96 Inhibitor Removal Plate	2 each
Collection Plate	2 each
Elution Plate	6 each
Foil Plate Cover	4 each
User manual	1 each

MSDS (Online: www.mpbio.com)	1 each
Certificate of Analysis	1 each

2.2 Storage

All FastDNA-96™ Soil Microbe DNA Kit reagents are stable at room temperature. Storage should be maintained at room temperature. The kit reagents are guaranteed for up to one year from the date of purchase of the kit.

2.3 User Supplied Materials

FastPrep-96™ Instrument (see Section 10)
Centrifuge with a swing-bucket style rotor that can spin up to 3,500 – 5,000 rpm
Swing-bucket centrifuge microplate adaptors for 96-well plates (pair)
Plate shaker
Sterile water

3. Important Considerations Before Use

3.1 Binding Plate Pre-Wash Buffer

The FastDNA-96™ Soil Microbe DNA Kit contains a bottle of Binding Plate Pre-Wash Buffer which may form a precipitate over time. The precipitate is easily resuspended in solution by gently warming the bottle at 30-37°C for up to 30 minutes with mixing by inversion. IMPORTANT: Do not microwave this reagent!

3.2 MP-96 Inhibitor Removal Plate Preparation

Prior to executing the protocol in Section 5, the MP-96 Inhibitor Removal Plate must be prepared for proper use in the assay. First mount the MP-96 Inhibitor Removal Plate on top of a supplied Elution Plate. Add 150 µl of Inhibitor Plate Prep Solution to the wells by puncturing the foil covering with the pipette tip. Incubate at room temperature for 5 minutes then centrifuge the stacked plates at 3,500 x g for 5 minutes. The MP-96 Inhibitor Removal Plate is now ready for sample preparation.

4. Safety Precautions

Some of the supplied kit reagents contain components that, when in contact with human tissue, may cause irritation. Wear personal protective equipment to prevent contact with the skin or mucus membranes (gloves, lab coat, and eye protection) at all times when using this product. Consult the Material Safety Data Sheet at www.mpbio.com for additional details. This product is for research purposes only.

5. Protocol

IMPORTANT NOTE BEFORE STARTING PROTOCOL:
When isolating DNA from soil samples, the wells of the MP-96 Inhibitor Removal Plate must be prepared for proper use. Do not attempt this assay without first preparing the MP-96 Inhibitor Removal Plate or humic substance contamination may occur. See Section 3.2, above, for instructions on preparing the MP-96 Inhibitor Removal Plate.

1. To the tubes of a FastDNA-96™ Lysing Matrix Rack, add up to 135 mg of soil and 400 µl of Lysis Buffer per well. Re-cap the tubes.

Optional: If broth culture cell lysis is desired, add 10-20 mg of wet weight bacterial or fungal cells, resuspended in up to 50 l of sterile water, or sterile PBS, to the tubes of the FastDNA-96™ Lysing Matrix Rack. Add Lysis Buffer and continue.

2. Load the FastDNA-96® Lysing Matrix Rack into the FastPrep-96™ Instrument, and process the samples. A single 60 second run at a speed setting of 1600 rpm is sufficient to lyse almost all samples. If additional processing time is required over 5 minutes, the FastDNA-96™ Lysing Matrix Rack should be incubated on ice for at least 2 minutes between successive runs to prevent overheating the samples.

3. Place the FastDNA-96™ Lysing Matrix Rack in a microplate centrifuge adaptor and spin at 3,500 -5,000 x g for 5 minutes.

NOTE: Extending centrifugation to 10 - 15 minutes can enhance elimination of excessive debris from soil samples, or from cells with complex cell walls.

4. Transfer up to 250 µl of supernatant to the wells of a clean Deep-Well Plate.

5. Add 750 µl of Soil DNA Binding Solution to the supernatant in each well of the Deep-Well Plate. Cover the wells of the Deep-Well Plate completely with the supplied Foil Plate Cover. Place the samples on a plate shaker or vortexer and shake/mix for 2 minutes.

6. Centrifuge the Deep-Well Plate for 5 minutes at 3,500 -5,000 x g.

7. Place the MP-96 Binding Plate on top of a supplied Collection Plate. Remove the foil from the Deep-Well Plate and transfer 500 µl of each supernatant to the wells of the MP-96 Binding Plate. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

8. Discard the flow-through from the Collection Plate and re-use. Repeat Step 7 until all of the supernatant has been carefully transferred to the binding plate.

9. Continue to re-use the Collection Plate by placing it beneath the MP-96 Binding Plate. To the wells of the MP-96 Binding Plate, add 200 µl of the Binding Plate Pre-Wash Buffer. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

10. To the wells of the MP-96 Binding Plate, add 500 µl of Soil DNA Wash Buffer.Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

11. Stack the MP-96 Binding Plate atop a clean Elution Plate. Add 50 - 100 µl of Elution Buffer directly to the matrix inside the wells of the MP-96 Binding Plate.Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

NOTE: DNA isolation is complete at this point if bacterial of fungal broth cultures were sampled. Continue with protocol only if sampling from soil or sludge.

12. Stack a prepared MP-96 Inhibitor Removal Plate (see Section 3.2 for details) atop a clean Elution Plate. Transfer the eluent from Step 11 to the wells of the MP-96 Inhibitor Removal Plate. Centrifuge the stacked plates for 5 minutes at 3,500 –5,000 x g.

13. Eluted DNA is now ready for PCR and other downstream applications. To store the samples, cover the Elution Plate with the supplied Foil Plate Cover. Store samples at 4°C until use, or at -20°C for extended periods.

6. Example Data: DNA Isolation from Soil Samples and Gel Electrophoresis

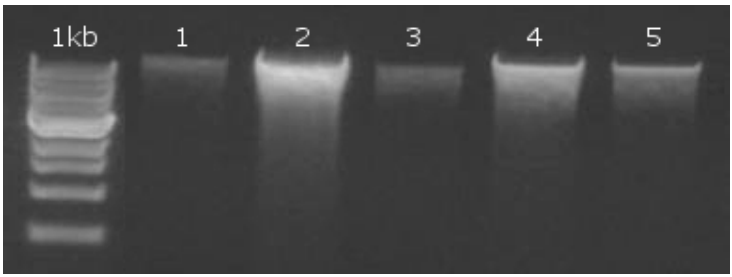

Figure 1.

DNA from various soil samples extracted with the FastDNA-96™ Soil Microbe Kit. 10% of the DNA isolated from 135 mg soil samples was loaded on a 0.8% agarose/ethidium bromide gel. (1 kb Ladder Marker, NEB)

Soil samples include:
Lane 1: Sand; Lane 2: Sandy Clay; Lane 3: Sandy Loam; Lane 4: Coarse Sandy Loam; Lane 5: Fine Gravel Sandy Loam. DNA ranges from 4-20 kb.

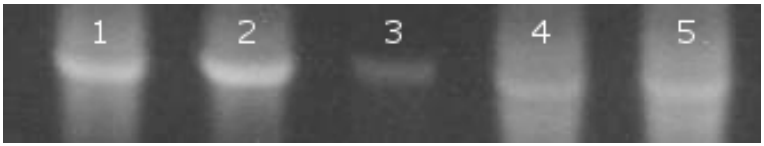

Figure 2.

PCR results of the DNA extractions from Figure 1, respectively. The PCR was executed using DNA primers specific to prokaryotic 16S rRNA.

7. Recommended Reference Format for Publications

DNA was isolated from (specific sample) using the FastDNA-96™ Soil Microbe DNA Kit and the FastPrep-96™ Instrument (MP Biomedicals, LLC, Santa Ana, CA).

8. References

Soil and Plant Laboratory, Inc. PO Box 117744, Santa Ana, CA 92711

9. Technical Support

For technical support with this product please contact our MP Biomedical's Technical Support Team at 1-800-854-0530, by email at biotech@mpbio.com, or visit us online at www.mpbio.com for live support.

For our European customers, please contact our European Technical Support Team at 00 800 7777 9999, or by email at techsup.eur@mpbio.com.

10. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative there of to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s). GeneClean® and FastPrep® are registered trademarks of MP Biomedicals, LLC.

Other FastPrep® Kits Available

FastDNA® Kit and FastDNA® Spin Kit
Cat N° 6540-400 - Cat N° 6540-600 respectively (100 preps)
- Lyse and isolate DNA in less than 30 minutes
- Plant, animal, yeast, fungal and microbial samples
- No hazardous organic reagents required
- SPIN filters streamline silica handling (FastDNA Spin Kit)

FastDNA® Spin Kit for Feces
Cat N° 6570-200 (50 preps)
- Lyse and isolate PCR-ready DNA in less than 30 minutes
- Variety of stool sample types
- No hazardous organic reagents required
- SPIN filters streamline silica handling

FastDNA® 50ml Spin Kit for Soil
Cat N° 6560-600 (10 preps)
- Process low-microbial containing soil samples.
- Lyse and isolate PCR-ready DNA in less than 60 minutes
- Variety of soil and environmental sample types
- No hazardous organic reagents required
- SPIN filters streamline silica handling

FastRNA® Pro Blue Kit
Cat N° 6025-050 (50 preps)
- For use with gram positive and gram negative bacteria
- Lyse up to 1010 cells per 2ml tube
- Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Red Kit
Cat N° 6035-050 (50 preps)
- For use with yeast cells and fungal tissue

• Lyse up to 1010 cells per 2ml tube
• Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Green Kit
Cat N° 6045-050 (50 preps)
• For use with all plant and animal samples
• Lyse 50-100 mg tissue per 2ml tube
• Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Soil-Direct Kit and FastRNA® Pro Soil-Indirect Kit
Cat N° 6070-050 - Cat N° 6075-050 respectively (50 preps)
• Isolate RNA from soil samples (direct kit) and washed soil (indirect kit) in less than 2 hours
• Variety of soil and environmental sample types
• RNA protected during and after processing
• Humic acids reduced to allow uninhibited RT-PCR
• Includes additional reagents for even further purification if necessary
• SPIN filters streamline silica handling

FastProtein™ Blue Matrix
Cat N° 6550-400 (50 preps) - Cat N° 6550-500 (100 preps)
• Release of proteins from gram positive and gram negative bacteria in 40 seconds
• Protein extracts are ready for immediate electrophoresis or purification
• Ideal for optimizing induction conditions

FastProtein™ Red Matrix
Cat N° 6550-600 (50 preps) - Cat N° 6550-700 (100 preps)
• Release of proteins from yeast cells and fungi in 40 seconds
• Protein extracts are ready for immediate electrophoresis or purification
• Ideal for optimizing induction conditions

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